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PRELIMINARY INVESTIGATION OF THE OCCURRENCE OF *LEGIONELLA* SPP. AND OTHER AQUATIC BACTERIA IN CHEMICALLY CONTAMINATED GROUND WATER TREATED BY AERATION

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16. ABSTRACT

Induced draft redwood slat aeration towers are used to remove volatile organic chemicals from ground water. This study looks at the effect of the aeration process upon the microbiological quality of the water. Preaeration and post-aeration water samples were examined for heterotrophic bacteria, total coliform bacteria and Legionella spp.

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PRELIMINARY INVESTIGATION OF THE OCCURRENCE OF LEGIONELLA SPP. AND OTHER AQUATIC BACTERIA IN CHEMICALLY CONTAMINATED GROUND WATER TREATED BY AERATION

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Ground water contaminated with volatile organic chemicals poses a major challenge to utilities which rely upon these waters as drinking water sources. Many of these volatile organic compounds, such as trichloroethylene, are suspected carcinogens. The inclusion of induced draft , redwood slat aerators in a water treatment train has been found to be an efficient and cost-effective method for the removal of these contaminants. This study was undertaken to evaluate the effect of the aeration process on the microbiological quality of the water. The similarity in design between the aeration towers and cooling towers, which have been previously implicated in outbreaks of Legionnaires' disease, provided the impetus for assaying for Legionella spp.

Water samples from an indoor aeration tower and an outdoor tower in New England were analyzed over a period of one and one-half years. Preaeration (raw ground water) and aerated samples were examined for the occurrence of Legionella spp., coliform bacteria and heterotrophic bacteria. Water temperatures for each sample were also recorded. Various culturing procedures, including direct plating, and acid pretreatment were utilized in assaying for Legionella spp.[4] using either buffered charcoal yeast extract agar, BCYE[5], a glycine-containing selective medium[7], or Edelstein's semi-selective medium, MWY[3]. All plates were incubated at 36 C and 3% CO_2. The inclusion of negative and positive control samples in each assay confirmed the adequacy of the methods used. Positive controls included spiked samples containing laboratory cultures of Legionella pneumophila, serogroup

1 and environmental samples from a hospital hot water tank known to contain L. pneumophila serogroups 1 and 5. Suspicious colonies, which morphologically resembled Legionella spp., isolated from the assays were subcultured on BCYE, BCYE minus cysteine and blood agar plates. Subcultures which grew only on BCYE were verified by the direct immunofluorescent technique[2] using fluorescein labeled antibody conjugates of L. pneumophila polyvalent (1,2,3,4) and monovalent conjugates 1 through 6. Conjugates for L. micdadei, L. gormanii, L. bozemanii, and L. dumoffii were also utilized. Organisms used as positive controls were similarly identified.

Heterotrophic bacteria were enumerated by the pour plate technique using standard plate count agar[1] and a low nutrient medium designated R2A (D. J. Reasoner and E. E. Geldreich, Abstr. Ann. Meet. Am. Soc. Microbiol. 1979, N7, p. 180). The R2A medium has been found to provide more representative profiles of the heterotrophic bacteria in water than those provided using conventional methodology. Analyses for total coliform bacteria were conducted by membrane filtration using M-Endo agar[1]. All sheen colony isolates from M-Endo were speciated using the API 20E system (Analytab Products, Plainview, N.Y.).

Levels of heterotrophic bacteria recovered during the various seasonal time periods of the study are shown in Table 1. The numbers of heterotrophic bacteria, with few exceptions, were consistently higher in the post-aeration samples, indicating possible colonization of the redwood slats.

Results from some individual samples showed dramatic increases in the number of bacteria in the post-aeration samples suggesting perhaps a sloughing off of the biomass on the redwood slats. A higher number of organisms was generally recovered using the R2A medium.

Total coliform bacterial levels (Table 2) were consistently low with the majority of the isolates coming from the postaeration samples. These results give further evidence that microbial colonization may be taking place in the aerators. The frequent isolation of Klebsiella spp. in the post-aeration samples is in keeping with previous studies reporting the association of this organism with redwood[6].

All cultural attempts to isolate Legionella spp. (Table 2) both from the water samples and from scrapings of the biomass from the redwood slats were negative. Analyses of the water samples by direct immunofluorescence also failed to elucidate any Legionella spp.

These results indicate that the use of aeration towers in drinking water treatment technology may be responsible for significantly higher levels of bacterial flora in the aerated water than that encountered in the raw water. Thus, indicating the need for a good postaeration disinfection program. These results further suggest that aeration towers may not pose a substantial risk for the introduction of Legionella spp. into the water supply. If Legionella organisms are present their numbers must be below the levels for detection using accepted procedures. It has been reported that Legionella spp. fail to multiply in aquatic environments below 25°C[7]. The lower temperatures characteristic of the ground waters studied ($\leq$ 12°C) may have been a major hindrance in the occurrence of this organism.

Further investigations are planned to study the microbial quality of water taken from the aeration tower located outdoors. These additional studies will investigate the influence of water hydraulics as a major cause of heterotrophic bacterial releases in the effluents.

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TABLE 1. Numbers of heterotrohphic bacteria isolated from raw ground water and aerated water from two New England aeration towers.

Date of Sample	Water Temperature °C		Plate Count Agar			R2A Medium		
			CFU per ml		Ratio Aerated:	CFU per ml		Ratio Aerated:
	Raw	Aerated	Raw	Aerated	Raw	Raw	Aerated	Raw
10-27-81	11.5	11.6	11	26	2.4	40	84	2.1
11-3-81	11.5	11.5	29	17	0.58	390	880	2.3
12-2-81	11.5	11.6	25	3,500	140.0	128	2,200	17.2
12-8-81	11.9	12.0	28	460	16.4	27	200	7.4
12-15-81	11.3	11.3	19	1,050	55.3	42	1,150	27.4
1-5-82	9.1	8.8	9	12	1.3	74	700	9.5
1-20-82	9.9	10.8	9	18,100	2,000.0	115	14,800	129.0
5-11-82	9.2	9.7	103	7	0.07	128	168	1.3
7-21-82	11.3	12.3	23	230	10.0	119	1,800	15.0
12-21-82	11.4	11.4	1	2	2.0	10	178	17.8
1-11-83	11.3	11.3	1	4	4.0	9	34	3.8
1-25-83	11.3	11.4	1	3	3.0	53	120	2.3
2-9-83	11.3	11.3	1	33	33.0	186	190	1.0
2-23-83	11.2	11.2	89	24	0.25	1,300	316	0.24
2-25-83	11.4	11.4	10	20	2.0	53	120	2.3
3-29-83	11.4	11.5	9	44	4.9	45	298	6.6
3-29-83	6.0	6.0	9	19	2.1	57	108	1.9
4-12-83	6.0	6.0	2,810	4,830	1.7	2,690	6,500	2.4
4-19-83	9.0	9.0	26	26	1.0	86	72	0.83

TABLE 2. Assays for total coliform bacteria and *Legionella* from raw ground water and aerated water from two New England aeration towers

Date of Samples	*Legionella* spp.* CFU/1 L		Total Coliforms CFU/100 ml		Species Identification (Isolates)	
	Raw	Aerated	Raw	Aerated	Raw	Aerated
10-27-81	< 1	< 1	< 1	< 1		
11-3-81	< 1	< 1	< 1	< 1		
12-2-81	< 1	< 1	< 1	< 1		
12-8-81	< 1	< 1	< 1	< 1		
12-15-81	< 1	< 1	1	1	*Enterobacter agglomerans*	*E. agglomerans*
1-5-82	< 1	< 1	< 1	< 1		
1-20-82	< 1	< 1	< 1	< 1		
5-11-82	< 1	< 1	< 1	1		*Citrobacter freundii*
7-21-82	< 1	< 1	< 1	10		*Klebsiella pneumoniae* (5) *K. oxytoca* (1) *C. freundii* (1) *Escherichia coli* (1) *Aeromonas hydrophila* (2)
12-21-82	< 1	< 1	< 1	< 1		
1-11-83	< 1	< 1	< 1	< 1		
1-25-83	< 1	< 1	NA	NA		
2-9-83	< 1	< 1	NA	NA		
2-23-83	< 1	< 1	< 1	< 1		
2-25-83	< 1	< 1	1	< 1	*E. agglomerans*	
3-19-83	< 1	< 1	< 1	1		*K. pneumoniae*
3-29-83	< 1	< 1	< 1	< 1		
4-12-83	< 1	< 1	< 1	< 1		
4-19-83	NA	NA	< 1	< 1		

NA = Not available

* Positive Controls: lyophilized *L. pneumophila*, serogroup 1, and environmental samples containing *L. pneumophila*, serogroups 1 and 5.